

EXHIBIT A

Product	Cat. No.	Size	Quantity	Price
20X SSC	15557-044	1 L	—	\$ 22.00
	15557-036	4 L	—	66.00
	15557-028	10 L	—	155.00

20X SSC (Ultra Pure) is a solution formulated for use in nucleic acid hybridizations and blot transfer applications. It is used in concentrations ranging from 0.2X to 20X, depending on the application. Supplied in 1-L plastic bottles, or in a 4-L or 10-L stackable CUBITAINER® Box, 20X SSC contains 3.0 M NaCl and 0.3 M sodium citrate, at pH 7.0.

Analytical Specifications

pH of 20X SSC at 23°C	7.0 ± 0.1
specific conductance of 20X SSC at 23°C	170 ± 10 mS/cm

Performance and quality testing: No detectable contaminating activity is observed in DNA nicking and ribonuclease assays.

Recommended storage condition: 15°C to 30°C.

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20X SSPE	15591-043	1 L	—	\$ 27.00
	15591-035	4 L	—	81.00
	15591-027	10 L	—	189.00

20X SSPE (Ultra Pure) is a prepared buffer concentrate formulated for use in nucleic acid hybridizations and blot transfer applications. Supplied in 1-L plastic bottles, or in a 4-L or 10-L stackable CUBITAINER® Box, 20X SSPE contains 3.0 M NaCl, 0.2 M NaH₂PO₄, and 0.02 M EDTA, at pH 7.4.

Analytical Specifications

pH of 20X SSPE at 25°C	7.4 ± 0.1
specific conductance of 20X SSPE at 25°C	180 ± 10 mS/cm

Performance and quality testing: No detectable contaminating activity is observed in DNA nicking and ribonuclease assays.

Recommended storage condition: 15°C to 30°C.

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Streptavidin Agarose	15942-014	5 ml	—	\$195.00
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For additional information about this product, please refer to page 14-9.

Sucrose	15503-014	1 kg	—	\$25.00
	15503-022	5 kg	—	69.00

Sucrose (Ultra Pure) is suitable for forming density gradients for a variety of separation applications. It is used to separate RNAs, to prepare bacteriophage λ DNA arms, and to separate proteins. Sucrose gradients typically are formed by use of gradient formers or by layering two or more sucrose solutions in varying amounts in a centrifuge tube.

Analytical Specifications

molecular weight	342.30
appearance	white, free-flowing crystals or powder
purity	≥99.9%
insolubles in a 50% (w/v) solution	none detected
lead	≤8 ppm
free glucose	≤0.1%

Performance and quality testing: No detectable contaminating activity is observed in protease assays.

Recommended storage condition: 15°C to 30°C, dry.

See also:

Gradient Formers, page 28-15.

Sucrose

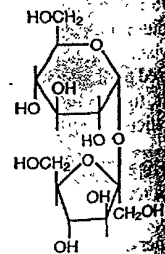


EXHIBIT B

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2. *Harsh treatment:* Pour several hundred milliliters of boiling 0.1% SDS onto the membrane. Cool to room temperature.

If a membrane is to be reprobbed, it must not be allowed to dry out between hybridization and stripping. If it becomes dry, the probe may bind to the matrix.

2. Place membrane on a sheet of dry Whatman 3MM filter paper and blot excess liquid with a second sheet. Wrap the membrane in plastic wrap and set up an autoradiograph.

If signal is still seen after autoradiography, rewash using harsher conditions.

3. The membrane can now be rehybridized. Alternatively, it can be dried and stored for later use.

Membranes can be stored dry between Whatman 3MM paper for several months at room temperature. For long-term storage, place the membranes in a desiccator at room temperature or 4°C.

REAGENTS AND SOLUTIONS

Aqueous prehybridization/hybridization (APH) solution

5× SSC (APPENDIX 2)

5× Denhardt solution (APPENDIX 2)

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see below) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Denatured salmon sperm DNA

Dissolve 10 mg Sigma type III salmon sperm DNA (sodium salt) in 1 ml water. Pass vigorously through a 17-G needle 20 times to shear the DNA. Place in a boiling water bath for 10 min, then chill. Use immediately or store at -20°C in small aliquots. If stored, reheat to 100°C for 5 min and chill on ice immediately before using.

Formamide prehybridization/hybridization (FPH) solution

5× SSC (APPENDIX 2)

5× Denhardt solution (APPENDIX 2)

50% (w/v) formamide

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see above) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Commercial formamide is usually satisfactory for use. If the liquid has a yellow color, deionize as follows: add 5 g of mixed-bed ion-exchange resin [e.g., Bio-Rad AG 501-X8 or 501-X8(D) resins] per 100 ml formamide, stir at room temperature for 1 hr, and filter through Whatman no. 1 paper.

CAUTION: Formamide is a teratogen. Handle with care.

Labeling buffer

200 mM Tris·Cl, pH 7.5

30 mM MgCl₂

10 mM spermidine

Mild stripping solution

5 mM Tris·Cl, pH 8.0

2 mM EDTA

0.1× Denhardt solution (APPENDIX 2)

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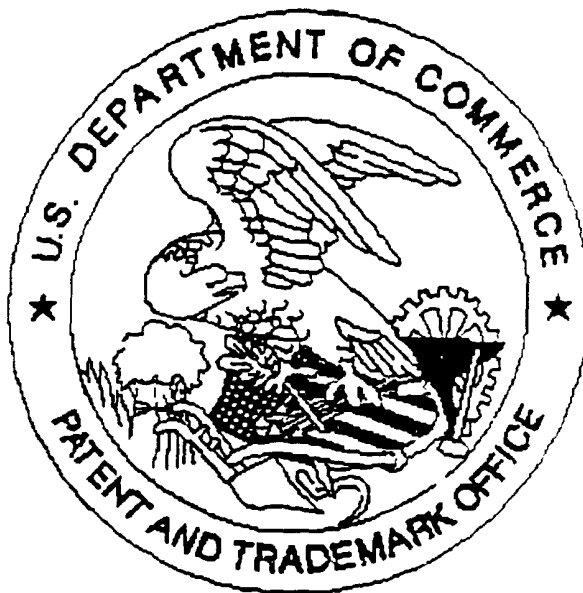
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